



## **Modification of gelatin functionality for culinary applications by using transglutaminase**

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## Scientific paper

Modification of gelatin functionality for culinary applications by using transglutaminase<sup>☆</sup>Julia Calvarro<sup>a</sup>, Trinidad Perez-Palacios<sup>a</sup>, Jorge Ruiz<sup>b,\*</sup><sup>a</sup> Food Science, Faculty of Veterinary Sciences, University of Extremadura, IPROCAR, UEX, Campus Universitario s/n, 10003 Cáceres, Spain<sup>b</sup> Department of Food Science; University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg C, Denmark

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## ABSTRACT

Porcine gelatin was subjected to the action of different amounts of commercial transglutaminase (TGase) and subsequently used to produce foams or gels. Foam stability at 20 °C and 80 °C, and thermal stability and instrumental texture of the gels were studied. Gelatin and TGase contents significantly increased the foam stability at both temperatures, but the effect of TGase was much more marked. Also both factors enhanced the thermal stability of gelatin gels, so that gels containing 3% of gelatin and 0.7% TGase were still gelled after 1 h at 80 °C, while any of the gelatin-based gels without TGase turned rapidly into liquid in less than 10 min at 80 °C. Hardness and chewiness of the gels were strongly enhanced by gelatin content, but very especially by TGase concentration. Gels tended to be less springy with increasing amounts of TGase. Modification of gelatin-based foams and gels with the addition of TGase appears as an interesting approach for culinary recipes in which gelatin should be heated. However, a careful optimization should be done to avoid a too rubbery texture.

## Introduction

Gelatin is a proteinaceous material obtained after the acid or basic hydrolysis of collagen, the major protein of animal connective tissue. Gelatin is commonly used as a gelling and foaming agent in many culinary preparations. Gels made with gelatin are very appreciated by chefs and consumers, due to their unique texture features: very soft and easily melting in the mouth before swallowing (Baziwane and He, 2003). Similarly, gelatin based foams are slightly viscous and consistent, showing a distinctive texture when tasted. However, gelatin melts at quite low temperatures: around 15 °C for fish gelatin and up to 35 °C for beef gelatin (Haug et al., 2004), and thus, gelatin-based foams and gels are not suitable for recipes involving heating, since gels would become liquid and foams would collapse in a short time. Moreover, the stability of gelatin-based foams is low even at room temperature, so that, once they have been prepared, they should be served and consumed within a short time. In fact, the use of other gelling agents, such as gellan gum, carrageenan or agarose, has become very popular as culinary ingredients, among other reasons, as a way to obtain gels and foams that can be heated (Lersch, 2014).

Transglutaminase (protein-glutamine  $\gamma$ -glutamyltransferase, EC 2.3.2.13) (TGase) catalyzes an acyl-transfer reaction between the  $\gamma$ -carboxamide group of peptide-bound glutamine residues and a variety of

amino acids. The use of TGase-catalyzed reactions to modify the functional properties of food proteins has been extensively studied (Ruiz-Carrascal and Regenstein, 2002). Gelatin is a good substrate for TGase, and therefore, the enzyme has been used to modify the properties of gelatin for biomedical and food applications (Fuchsbauer et al., 1996). In fact, gelatin films with a noticeably high thermal stability have been obtained by incubation with TGase (Yi et al., 2006). Some authors (Kołodziejaska et al., 2004) found a substantially lower setting time when incubating gelatin solutions with TGase, and also a lower amount of gelatin needed for obtaining a gel. However, some of the physical features of the gelatin are modified when enzymatically treated with TGase (Chiou et al., 2006).

In the present study we hypothesised that the stabilization of gelatin based foams and gels by incubation with TGase could be an interesting approach for obtaining thermally stable gelatin-based foams and gels for culinary recipes. Moreover, we also addressed how the texture characteristics of the gelatin gels were modified as a consequence of the effect of TGase.

## Material and methods

## Experimental design

Two different experiments were performed:

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### Experiment 1: stability of gelatin-based foams under heat and room temperature as affected by TGase

Gelatin foams were prepared by mixing different amounts of porcine gelatin (80–100 bloom, Panreac, Spain) (1%, 2% and 3%, w/v) in distilled water and different amounts of commercial transglutaminase (TGase) (Activa EB, Ajinomoto, Japan) (0%, 0.35% and 0.7%, w/v) at room temperature. Each combination gelatin × TGase was repeated 5 times. The solutions were then poured into a siphon canister, sealed and pressurized with two charges of propellant gas (N<sub>2</sub>O), vigorously shaken and let setting for 12 h. Afterwards, the siphon canister was shaken again and foams were siphoned in crystal pots and stored either at room temperature or at 80 °C, and foam stability was measured.

### Experiment 2: Texture and stability of gelatin-based gels as affected by TGase

Gelatin gels were prepared by mixing different amounts of porcine gelatin (2%, 2.5% and 3%, w/v) and different amounts of commercial TGase (0%, 0.35% and 0.7%, w/v) in distilled water. Gelatin was dissolved in hot water (80 °C); solutions were then cooled at room temperature. A solution with the appropriate amount of TGase was added when the temperature of the solution was at 40 °C (100% of TGase activity remains at 40 °C). In order to counteract the potential effect of TGase itself (as a protein) or maltodextrin (major component of Activa EB, the commercial TGase used in this study) on the gel properties, all the solutions included the appropriate amount of inactivated Activa EB (heated solution of Activa EB at 90 °C) to complete a total concentration of Activa EB of 0.7% (w/v). In 0% TGase samples all the Activa EB was inactivated, while in 0.7% TGase ones all the existing Activa EB was active. 10 mL of the solutions containing gelatin and TGase were transferred to Petri dishes and let setting at room temperature and then cooled at 4 °C for 24 h, giving rise to 1 cm thickness gelatin gels. Each combination gelatin × TGase was repeated 5 times. The instrumental texture and thermal stability of gelatin-based gels was measured in all samples.

### Foam stability

Foam stability was assessed in 5 samples by measuring the time that it takes the collapse of the foam column to half of its initial value (Bouaouina et al., 2006).

### Gel thermal stability

The thermal stability of gelatin-based gels was evaluated in 5 samples following the method suggested by Kołodziejaska et al. (2004): petri dishes containing the gels were incubated at 80 °C and the physical state of the gel was visually controlled every 10 min until melting.

### Instrumental texture

Texture analyses were performed in a texturometer TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK). For the determination of the texture profile analysis (TPA), uniform portions of the gels were cut into 1 cm<sup>3</sup> cubes (*n*=5 for each treatment). They were axially compressed to 50% of the original height with a flat plunger of 50 mm in diameter (P/50) at a crosshead speed of 2 mm s<sup>-1</sup> through a 2-cycle sequence. The following texture parameters were measured from force deformation curves (Bourne, 1978): Hardness (N) = maximum force required to compress the sample (peak force during the first compression cycle); Springiness (cm) = height that the sample recovers during the time that elapses between the end of the first compression and the start of the second; Cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required to for the first compression and A2 the total energy required for the second compression); Chewiness

(N cm) = the work needed to chew a solid sample to a steady state of swallowing (hardness × cohesiveness × springiness).

### Statistics

An individual gel or foam was the experimental unit. The effects of the proportion of gelatin (1%, 2% and 3% for the foams and 2%, 2.5% and 3% for the gels) and TGase (0%, 0.35% and 0.7% of commercial TGase) were analyzed by a two-way analysis of variance together with their interaction (gelatin × TGase), using the GLM procedure (SPSS 15.0). The Tukey's test was used at the 5% level to make comparisons between sample means when pertinent.

## Results and discussion

### Foam stability

Fig. 1 shows the average stability of gelatin-based foams with different amounts of TGase at room temperature. As a general trend, both the increase in gelatin and in TGase content caused a better foam stability (*p* < 0.001 for both factors). There was a significant interaction between gelatin and TGase (*p* < 0.001). In fact, it can be clearly observed that while the stability of TGase free foams increased progressively with the amount of gelatin (dark bars in the figure), in those containing either 0.35% or 0.7% commercial TGase, there was a marked shift in the stability from 1% to 2% gelatin, while it remained similar from 2% to 3% (grey and white bars).

Gelatin is a reasonably good foaming agent, and with this purpose it is used for example in baking or in the production of marshmallows (Baziwane and He, 2003). Chefs very frequently use gelatin for thick foams aimed to be served cold. As compare with other foaming agents, gelatin-based foams show a very smooth mouth feel, with small and numerous bubbles, that falls within the group of the so-called “mousses”.

It is known that the stability of gelatin foams increases with the amount of gelatin, unless within the range of concentrations used in this study (Hafidz and Yaakob, 2011). This is in agreement with our findings, since we showed a clear increase in the stability with higher proportion of gelatin in the gel. Interestingly, the increase in the amount of TGase very much improved the foaming properties of TGase. Previous studies have pointed out the modification of the functional properties of gelatin through TGase, but to our knowledge this is the first in which foaming stability of TGase modified gelatin is addressed. This effect could be partially due to the more stable structure of the protein network in the bubble walls due to the achieved protein crosslinking. However, other authors have observed a decrease

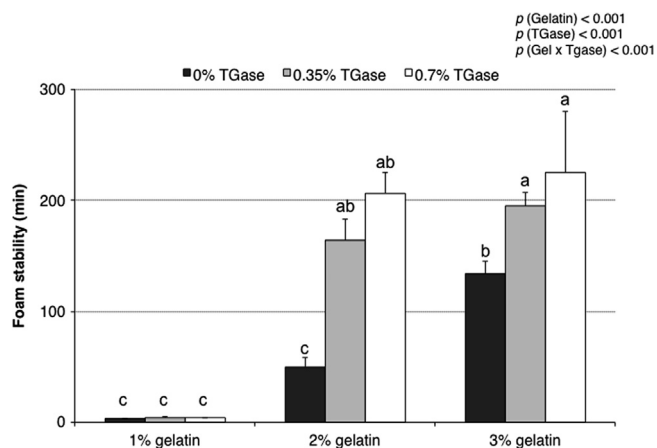
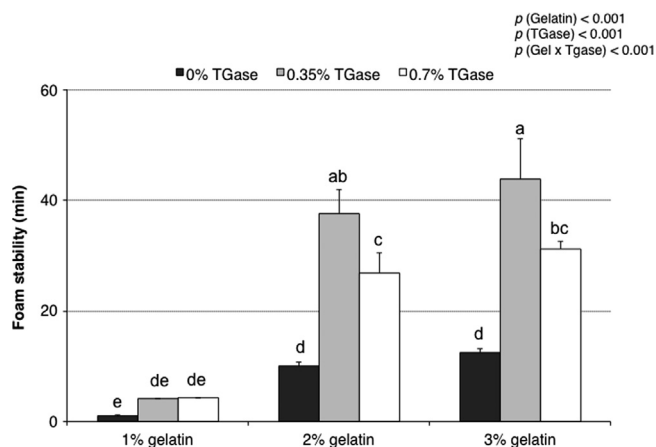


Fig. 1. Stability (mins) at room temperature (approx. 20 °C) of foams made with different amounts of gelatin and TGase. Values are the average of 5 samples plus SD. Statistical significances of the effects are shown in the upper right corner. Bars with different letters show significant differences in the Tukey's test (*p* < 0.05).



**Fig. 2.** Stability (mins) at 80 °C of foams made with different amounts of gelatin and TGase. Values are the average of 5 samples plus SD. Statistical significances of the effects are shown in the upper right corner. Bars with different letters show significant differences in the Tukey's test ( $p < 0.05$ ).

in the foaming properties of other proteins as a result of the incubation with TGase (Flanagan et al., 2003), so that the potential effect of crosslinking on foam stability might vary from protein to protein. At any rate, within the range of gelatin used in this study, the use of TGase could be an interesting approach for stabilizing culinary foams, allowing the preparation of the course a longer time before the serving, which could be specially useful in catering or central kitchens.

Both gelatin and TGase contents and their interaction showed a significant ( $p < 0.001$ ) effect on the stability of gelatin-based foams at 80 °C (Fig. 2) but this effect was much more marked for TGase, multiplying by a factor of 3 or more the stability of 2% and 3% gelatin foams.

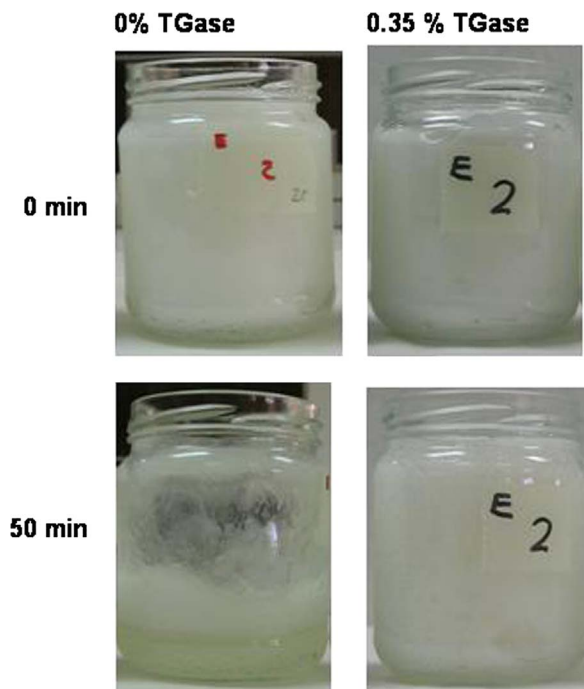
Incubation of TGase free gelatin foams at 80 °C led to rapid foam collapse, regardless the gelatin content, evidencing the unfeasibility of gelatin as a foaming agent when the mousses or foams are aimed to be heated. However, through the incubation with TGase, considerably higher foam stability was achieved, which will potentially allow such kind of recipes. Fig. 3 shows such an effect: Pots containing gelatin-based foams (2% w/v) either incubated or not with TGase (0.35%) after heating at 80 °C for 50 min

Curiously, the stability was higher when using 0.35% of TGase, but there was a significant ( $p < 0.05$ ) decrease in foam stability from 0.35% to 0.7% TGase, although the stability was still higher than without the enzyme. In fact, when observing the evolution of foams upon heating, the first minutes, foam volume growth, and the upper surface curved outwards, due to an increase in the volume of the bubbles as a consequence of the temperature. The interesting question is that gelatin foams treated with TGase resisted such an “inflation” process for sometime, while untreated ones failed. Thus, enzymatic modification of gelatin with TGase appears as a procedure that allows the use of gelatin foams in heated culinary preparations.

Thermal stability of the foams also increased with a higher gelatin content in absence of TGase. The increase from 1% to 2% gelatin was much more marked than from 2% to 3%, due to the total instability of 1% gelatin foams. Nevertheless, and considering that foam stability is defined in this study as the time to reach 50% of the original foam height, the stability achieved was most likely insufficient for real applications even at 3% gelatin.

#### Thermal stability of gels

Table 1 shows the physical state of gels with different concentrations of gelatin and incubated with or without TGase and kept at 80 °C for different times. Gelatin gels made without added TGase became liquid in less than 10 min at 80 °C regardless the gelatin concentrations. This was of course expected due to the melting point of gelatin,



**Fig. 3.** Gelatin foams (2% w/v) with or without TGase after incubation at 80 °C for 50 min

which in the case of porcine gelatin is around 30 °C (Haug et al., 2004), and evidences that gelatin gels are not suitable for culinary recipes that should be served hot. Incubation of gelatin with TGase led to enhanced thermal stability of the gel. Thus, within any of the checked gelatin concentrations, the use of TGase led to a marked shift in the time it took for the gel to become liquid, so that at the same heating time as those in which they were already liquid without TGase, the gels became either a sol (texture similar to a thick jam) or continued as gels when containing TGase. In fact, 3% (w/v) gelatin gels with 0.7% TGase remained gelled even after 60 min at 80 °C.

The effect of TGase on the thermal stability of gelatin-based gel is well known, and has been used as a way to improve the mechanical properties of this type of gels for medical applications (Mcdermott et al., 2004). Other studies have previously described the effect of TGase in increasing the melting point of gelatin during industrial processing of collagen from different species. Thus, Kołodziejaska et al. (2004) found that TGase was an effective strategy to increase the melting point of industrially produced gelatin from fish skins. Moreover, such a strategy has also been used to improve the functionality of gelatin-based films (Chambi and Grosso, 2006).

Fig. 4 shows a mint flavoured tea gel made with gelatin and stabilized through the use of TGase, prepared by the chef (Galiano, 2006). This gel was microwaved and did not melt and, as shown in the pictures, was thereafter directly subjected to a flame to a point that even promoted the caramelization and burning of the surface. This effect could not have been achieved with regular gelatin gels, since it would have rapidly melted due to the action of the flame and the low melting point of the gelatin.

Nevertheless, TGase affected some of the features of the gelatin-based gels. For example, gels were less transparent and more turbid, probably due to the formation of a stronger and chemically stabilised protein network (Yi et al., 2006). Moreover, texture of gels was also affected, but this will be discussed in next section.

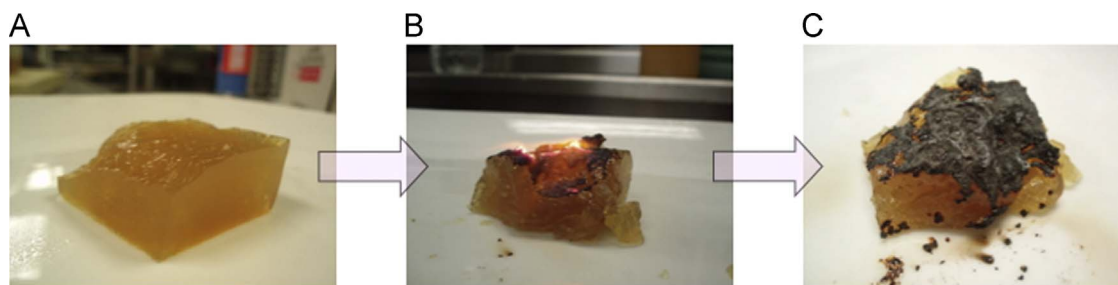
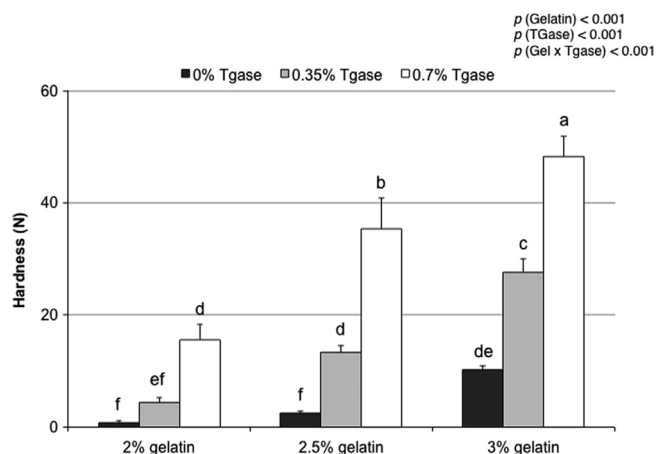
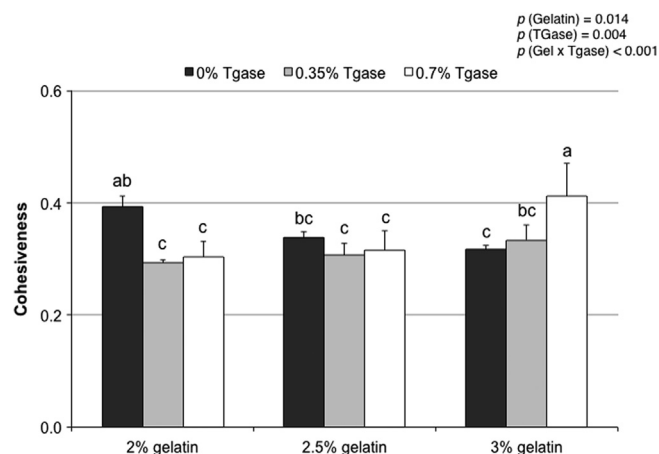
#### Instrumental texture of gels

Figs. 5–8 show the hardness, cohesiveness, springiness and chewiness of gels made with different amounts of gelatin and incubated with

**Table 1**

Physical state of gelatin solutions incubated with different amounts of TGase after heating at 80 °C for different times.

% gelatin	% TGase	Incubation time at 80 °C						
		0 min	10 min	20 min	30 min	40 min	50 min	60 min
2	0	Sol <sup>a</sup>	Liq <sup>b</sup>	Liq	Liq	Liq	Liq	Liq
2	0,35	Gel <sup>c</sup>	Liq/Sol	Liq/Sol	Liq/Sol	Liq/Sol	Liq/Sol	Liq/Sol
2	0,7	Gel	Gel	Sol/Gel	Sol/Gel	Sol	Liq/Sol	Liq/Sol
2,5	0	Sol	Liq	Liq	Liq	Liq	Liq	Liq
2,5	0,35	Gel	Gel	Liq/Gel	Liq/Sol	Liq/Sol	Liq/Sol	Liq/Sol
2,5	0,7	Gel	Gel	Gel	Gel	Liq -Gel	Liq -Gel	Liq -Gel
3	0	Gel	Liq	Liq	Liq	Liq	Liq	Liq
3	0,35	Gel	Gel	Sol/Gel	Sol	Sol	Sol	Sol
3	0,7	Gel	Gel	Gel	Gel	Gel	Gel	Gel

<sup>a</sup> Sol: Very thick solution, showing a texture between thick jam and egg white.<sup>b</sup> Liq: Totally liquid solution, flowing easily when poured out of the dish.<sup>c</sup> Gel: the original gel keeps the shape and it is not pourable and remains stuck to the dish.**Fig. 4.** Mint flavoured tea gelatin stabilised through the action of TGase (A), subjected to the direct action of a flame (B), leading to an intense burning of the surface but avoiding melting (C). Taken from Galiano (2006) with permission.**Fig. 5.** Hardness (N) in an instrumental texture profile analysis of gels made with different amounts of gelatin and TGase. Values are the average of 5 samples plus SD. Statistical significances of the effects are shown in the upper right corner. Bars with different letters show significant differences in the Tukey's test ( $p < 0.05$ ).**Fig. 6.** Cohesiveness (dimensionless) in an instrumental texture profile analysis of gels made with different amounts of gelatin and TGase. Values are the average of 5 samples plus SD. Statistical significances of the effects are shown in the upper right corner. Bars with different letters show significant differences in the Tukey's test ( $p < 0.05$ ).

different amounts of commercial TGase, in a texture profile analysis (TPA) test. Hardness is related to the strength of the gel structure under compression, and was significantly affected by the proportion of gelatin and by the amount of TGase ( $p < 0.001$  for both). Both of those factors showed a positive effect on the hardness of the gel, so that the higher the amount of gelatin and of TGase, the harder the gel was. Moreover, there was also a significant interaction between both factors ( $p < 0.001$ ), so that the positive effect of TGase on hardness was more marked with higher amounts of gelatin.

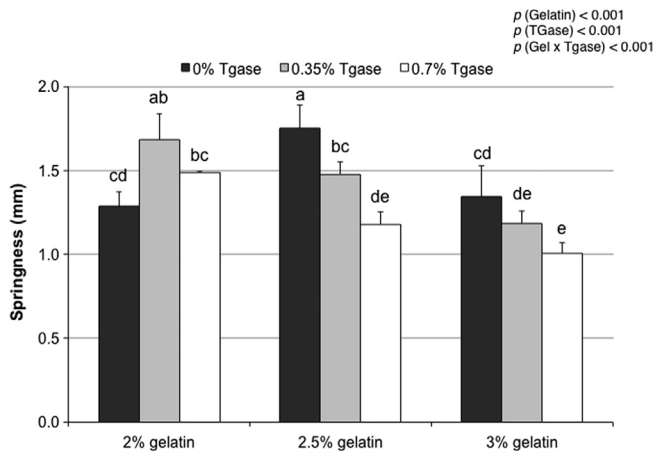
Cohesiveness is a measure of the degree of difficulty in breaking down the gel's internal structure. Even though this parameter was also significantly affected by both studied factors, the effects were not as clear as for hardness. Basically, it seems that the gels containing 3%

gelatin became more cohesive with higher amounts of TGase, while the opposite behaviour was observed for 2% gelatin gels, and no significant effect of TGase on the 2.5% gelatin gels was detected.

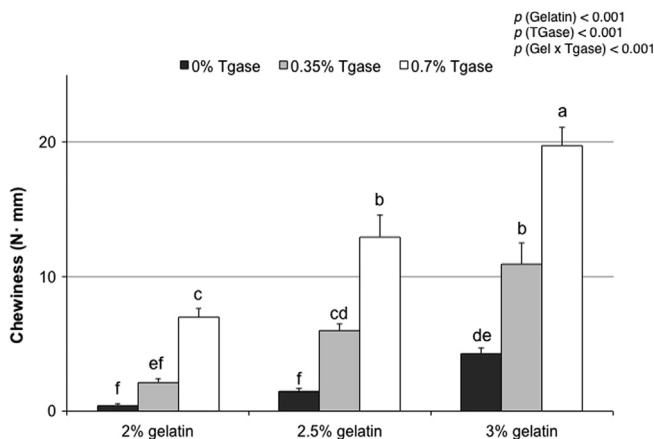
Springiness is somehow related to the rubbery felling when a gel is chewed, and it is a measure of how much the gel structure is broken down by the initial compression. Even though gelatin, TGase and their interaction showed a significant effect on this parameter ( $p < 0.001$  for all of them), as for the cohesiveness, these effects were a bit unclear. Thus, while for 2.5% and 3% gelatin gels, TGase produced a decrease in springiness, for 2% gelatin gels, incubation with TGase led to increased springiness.

Finally, chewiness reflects the energy required to masticate a solid food to a state ready for swallowing. Both, the amount of gelatin and of





**Fig. 7.** Springiness (mm) in an instrumental texture profile analysis of gels made with different amounts of gelatin and TGase. Values are the average of 5 samples plus SD. Statistical significances of the effects are shown in the upper right corner. Bars with different letters show significant differences in the Tukey's test ( $p < 0.05$ ).



**Fig. 8.** Chewiness (N·mm) in an instrumental texture profile analysis of gels made with different amounts of gelatin and TGase. Values are the average of 5 samples plus SD. Statistical significances of the effects are shown in the upper right corner. Bars with different letters show significant differences in the Tukey's test ( $p < 0.05$ ).

TGase, significantly ( $p < 0.001$  for both of them), increased the chewiness of the gel. Moreover, there was a significant ( $p < 0.001$ ) and clear interaction between both factors, so that the effect of higher amounts of TGase was much more marked in gels containing higher proportions of gelatin.

There are two different interesting aspects to be discussed around the texture profile analysis test: On one hand, the plausible explanation of the obtained results based on the effects of gelatin and TGase on the texture of gels; on the other hand, the potential practical outcome of the use of TGase as a modifier of gelatin gels properties.

The formation of a regular gelatin gel involves that protein coils assemble into triple helices upon cooling, so that a protein network is formed giving rise to a gel (Giraudier et al., 2004). In a TGase modified gelatin, on top of such mechanisms, there happens the formation of chemical bonds between protein chains, also leading to the formation of a network. Both types of network formation have consequences on the viscoelastic properties of the gel, so that the creation of a more rigid and stable chemical network, clearly influences the final physical properties of the gelatin-based gel, and thus its texture.

Gelatin modified gels were harder, chewier and less elastic. This could lead, in the more extreme situations, to a gel showing a

disastrous mouth feeling, with numerous small pieces of gel upon chewing, rather than the characteristic melting in the mouth effect of gelatin. Nevertheless, the combination of different proportions of gelatin with different amounts of TGase allowed obtaining a wide range of texture features. The optimization of gelatin  $\times$  TGase combination for achieving thermal stable gels should consider not only the thermal stability, but a deep study of the effects on the texture parameters, that should be thereafter confirmed and further investigated with sensory analysis. It should be not forgotten that the use of different gelatin blooms and modifiers, such as sodium chloride, fat, sugar and so on, could also modify the features of the gel. From a practical point of view, 0.7% TGase gels tended to be too hard and chewy, so that the potential advantages of having a thermal stable gel are meaningless, since the texture of the gel is very much impaired, and far chewier than what a chef is expecting for most culinary applications. However, it is clear from the results of the study that by playing with the amount of TGase and gelatin, it is feasible to find a balance in which the thermal stability is very much improved, and the texture is not yet significantly affected.

## Conclusions

An optimization of the proportion of gelatin and TGase, and perhaps, the use of compounds that may affect the texture of gelatin gels, such as fat, salt or sugars, is necessary before the use of TGase as a method for modifying gelatin gels for culinary purposes. Nevertheless, modification of gelatin properties through the use of TGase appears as an interesting approach for obtaining thermally resistant gelatin gels and foams.

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